

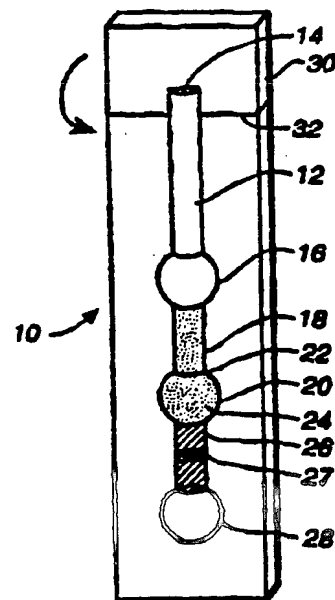
PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|-----------|---|
| (51) International Patent Classification ⁵: B01L 3/00, C12Q 1/68 | A1 | (11) International Publication Number: WO 94/26414 (43) International Publication Date: 24 November 1994 (24.11.94) |
| (21) International Application Number: PCT/US94/04849 (22) International Filing Date: 3 May 1994 (03.05.94) (30) Priority Data: 08/062,885 17 May 1993 (17.05.93) US (71) Applicant: SYNTEX (U.S.A.) INC. [US/US]; 3401 Hillview Avenue, Palo Alto, CA 94304 (US). (72) Inventors: ULLMAN, Edwin, F.; 135 Selby Lane, Atherton, CA 94025 (US). IRVIN, Benjamin, R.; 1779 Silocci Drive, Campbell, CA 95008 (US). (74) Agents: CHOW, Y., Ping et al.; Syntex (U.S.A.) Inc., 3401 Hillview Avenue, Palo Alto, CA 94304 (US). | | (81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |

(54) Title: REACTION CONTAINER FOR SPECIFIC BINDING ASSAYS AND METHOD FOR ITS USE**(57) Abstract**

Devices and methods for carrying out an assay for the determination of nucleic acids are disclosed. The devices comprise (a) a sample receiving chamber, (b) a plurality of additional chambers, at least one of the additional chambers containing reagents for carrying out an assay for the determination of a nucleic acid, (c) means for detecting a signal generated by the reagents, (d) means for permitting fluid communication between the additional chambers, (e) means for introducing a sample into the device, and (f) means for separating the analyte from the remainder of the sample and introducing the analyte into one of the additional chambers, which means comprises suspendible particles. The methods involve introducing a sample suspected of containing a nucleic acid analyte into a device in accordance with the invention. The analyte, if present, and particles in a medium are transported through the chambers of the device. Reagents for conducting an amplification are combined with the transported medium, which is subjected to conditions for amplifying the analyte. The medium is then examined for the presence of amplified analyte.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

REACTION CONTAINER FOR SPECIFIC
BINDING ASSAYS AND METHOD FOR ITS USE

BACKGROUND OF THE INVENTION

1. Field of the Invention.

This invention relates to reaction containers in which nucleic acids are amplified and detected without exposing the nucleic acid to the environment or the environment to amplified nucleic acid.

Nucleic acid hybridization has been employed for investigating the identity and establishing the presence of nucleic acids. Hybridization is based on complementary base pairing. When complementary single stranded nucleic acids are incubated together, the complementary base sequences pair to form double stranded hybrid molecules. The ability of single stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (RNA) to form a hydrogen bonded structure with a complementary nucleic acid sequence has been employed as an analytical tool in molecular biology research. The availability of radioactive nucleoside triphosphates of high specific activity and the ³²P labelling of DNA with T4 kinase has made it possible to identify, isolate, and characterize various nucleic acid sequences of biological interest. Nucleic acid hybridization has great potential in diagnosing disease states associated with unique nucleic acid sequences. These unique nucleic acid sequences may result from genetic or environmental change in DNA by insertions, deletions, point mutations, or by acquiring foreign DNA or RNA by means of infection by bacteria, molds, fungi, and viruses. Nucleic acid hybridization has, until now, been employed primarily in academic and industrial molecular biology laboratories. The application of nucleic acid hybridization as a diagnostic tool in clinical medicine is limited because of the frequently very low concentrations of disease related DNA or RNA present in a patient's body fluid or tissue and the unavailability of a sufficiently sensitive method of nucleic acid hybridization analysis.

Recently, a method for the enzymatic amplification of specific double stranded sequences of DNA known as the polymerase chain reaction (PCR) has been described. This in vitro amplification procedure is based on repeated cycles of denaturation, oligonucleotide primer annealing, and primer extension by thermophilic polymerase, resulting in the exponential increase in copies of the desired sequence flanked by the primers. The two different PCR primers, which anneal to opposite strands of the DNA, are positioned so that the polymerase catalyzed extension product of one primer can serve as a template strand for the other, leading to the accumulation of a discrete double stranded fragment whose length is defined by the distance between the 5' ends of the oligonucleotide primers.

-2-

Another method that has recently been described is an amplification of a single stranded polynucleotide using a single polynucleotide primer. The single stranded polynucleotide that is to be amplified contains two non-contiguous sequences that are complementary to one another and, thus, are capable of hybridizing together to form a stem-loop structure. This single stranded polynucleotide may be already part of a polynucleotide analyte or may be created as the result of the presence of a polynucleotide.

Another method for achieving the result of an amplification of nucleic acids is known as the ligase chain reaction (LCR). This method uses a ligase enzyme to join preformed nucleic acid probes. The probes hybridize with the nucleic acid analyte, if present, and ligase is employed to link the probes together resulting in two templates that can serve in the next cycle to reiterate the particular nucleic acid sequence.

Another method for achieving a nucleic acid amplification is the nucleic acid sequence based amplification (NASBA). This method is a primer-directed, enzymatic process that induces in vitro continuous, homogeneous and isothermal amplification of a specific nucleic acid.

Another method for amplifying nucleic acids is the Q-beta-replicase method, which relies on the ability of Q-beta-replicase to amplify a specific RNA substrate exponentially and is used as a label to detect binding rather than a method to create more target nucleic acid.

One method for detecting nucleic acids is to employ nucleic acid probes. One method utilizing such probes is described in U.S. Patent No. 4,868,104, the disclosure of which is incorporated herein by reference. A nucleic acid probe may be, or may be capable of being, labeled with a reporter group or may be, or may be capable of becoming, bound to a support.

Detection of signal depends upon the nature of the label or reporter group. If the label or reporter group is an enzyme, additional members of the signal producing system would include enzyme substrates and so forth. The product of the enzyme reaction is preferably a luminescent product, or a fluorescent or non-fluorescent dye, any of which can be detected spectrophotometrically, or a product that can be detected by other spectrometric or electrometric means. If the label is a fluorescent molecule, the medium can be irradiated and the fluorescence determined. Where the label is a radioactive group, the medium can be counted to determine the radioactive count.

Until recently, the amplification of nucleic acids has been carried out in stoppered containers to obtain the desired number of copies. Then, the container is opened and the amplification medium is withdrawn and transferred to a detection apparatus. Alternatively, the reagents used for detection are added to the container used for the amplification and the detection is carried out in the same container.

Such a technique is unsatisfactory for convenient and widespread use of amplification technology because aerosols are produced in the act of opening the containers and during the transfer of fluids. Such aerosols contain a few molecules of the amplified nucleic acid, which are released into the environment. Normally, such few molecules in the environment are not of great concern. However, only one molecule is needed to contaminate other containers that are to be used in further amplifications. This possibility for contamination can result in a false test since such a single molecule can be amplified and detected. The result of the test will not accurately reflect the presence or absence of the particular nucleic acid in the patient sample being tested.

Recently, a containment cuvette for amplification of nucleic acids has been disclosed. The cuvette and its method of use are designed to prevent amplified nucleic acid from being released into the atmosphere.

The need still exists for devices and methods for carrying out assays that avoid false positives caused by cross-contamination of samples, avoid handling of liquid reagents, are preferably homogeneous and are automatable with relatively simple instrumentation.

2. Description of the Related Art

A containment cuvette for conducting PCR is disclosed in European Patent Application publication number 0 381 501 (Schnipelsky, *et al.*). Detection reagents are either pre-incorporated into compartments in the cuvette or are added after amplification. In the latter situation a check valve prevents amplified nucleic acid from being released. Transfer of liquids between compartments is achieved by the use of flexible compartment walls and an external pressure source or by pistons that are part of the cuvette and operate on the compartments as a piston within a piston chamber.

A device for processing biological specimens for analysis of nucleic acids is described in U.S. Patent No. 5,188,963. The device has a hinged compartment facilitating automation of DNA- and RNA-based diagnostics and genetic surveillance and detection. Specimens are embedded in a matrix in the carrier. The matrix is then treated by one or more of the techniques such as amplification, electrophoresis, and hybridization as selected for the desired analysis and then the sample is treated to detect the cellular component.

A process for amplifying, detecting and/or cloning nucleic acid sequences otherwise referred to as PCR is disclosed in U.S. Patent Nos. 5,008,182, 4,965,188, 4,800,159, 4,683,195 and 4,683,202. Sequence polymerization by PCR is described by Saiki, *et al.*, (1986) *Science*, 230: 1350-1354.

U.S. Patent Applications Serial Nos. 07/299,282 and 07/399,795, filed January 19, 1989, and August 29, 1989, respectively, describe nucleic acid amplification using a single polynucleotide primer. U.S. Patent

Application Serial No. 07/555,323 filed July 19, 1990, discloses methods for producing a polynucleotide for use in single primer amplification. U.S. Patent Application Serial No. 07/555,968 filed July 19, 1990, describes a method for producing a molecule containing an intramolecular base-pair structure. U.S. Patent Application Serial No. 07/776,538 filed October 11, 1991, discloses methods for producing a polynucleotide for use in single primer amplification. U.S. Patent Application Serial No. 07/923,079 filed July 31, 1992, describes a method for introducing sequences at the 3' end of polynucleotides. The disclosures of these six applications are incorporated herein by reference in their entirety.

Other methods of achieving the result of a nucleic acid amplification are described by Van Brunt in *Bio/Technology* (1990) 8 (No.4):291-294. These methods include ligase chain reaction (LCR), nucleic acid sequence based amplification (NASBA) and Q-beta-replicase amplification of RNA. LCR is also discussed in European Patent Applications Nos. 439,182 (Backman I) and 473,155 (Backman II).

Methods for separating components in a mixture employing magnetic particles and a second medium are described in U.S. Patent Application Ser. No. 07/455,550 filed December 22, 1989 (Vorpahl), the disclosure of which is incorporated herein by reference.

One particular method that may be applied in the detection of amplified nucleic acid is described in U.S. Patent Application Ser. No. 07/704,569 filed May 22, 1991 (Ullman, *et al.*), the relevant disclosure of which is incorporated herein by reference. The method comprises treating the reaction mixture suspected of containing the amplified nucleic acid analyte under conditions such that the analyte, if present, causes a photosensitizer and a chemiluminescent compound to come into close proximity. The photosensitizer generates singlet oxygen and activates the chemiluminescent compound when it is in close proximity. The activated chemiluminescent compound subsequently produces light. The amount of light produced is related to the amount of analyte in the medium.

SUMMARY OF THE INVENTION

The present invention includes devices and methods for using the devices for conducting amplifications of nucleic acids. One device in accordance with the present invention comprises (a) a sample receiving chamber, (b) a plurality of additional chambers, at least one of the additional chambers containing reagents for carrying out an assay for the determination of a nucleic acid, (c) means for detecting a signal generated by the reagents, (d) means for permitting fluid communication between the additional chambers, (e) means for introducing a sample into the device, and (f) means for separating the nucleic acid analyte from the remainder of the sample and introducing the nucleic acid analyte into one of the

-5-

additional chambers, which means comprises suspendible particles.

Another embodiment in accordance with the present invention is a device for amplifying and detecting a nucleic acid analyte comprising: (a) a port for introducing a sample suspected of containing a nucleic acid analyte into the device, (b) a first chamber, for receiving the sample, in fluid communication with the port, wherein fluid communication between the port and the first chamber is capable of being sealed off, (c) a channel, containing a liquefiable gel, in fluid communication with the first chamber, (d) a second chamber containing reagents for amplifying the nucleic acid analyte, wherein fluid communication between the second chamber and the channel is prevented by a temporary seal, and (e) a third chamber, wherein fluid communication between the second chamber and the third chamber is prevented by a temporary seal.

Another embodiment in accordance with the present invention is a method for amplifying and detecting a nucleic acid analyte using a sealable device. The method comprises the steps of: (a) introducing a sample suspected of containing the nucleic acid analyte into a first chamber of the device preceding or following combining the sample with particles capable of binding the nucleic acid analyte, (b) transporting the particles from the first chamber through a channel containing air or a liquefiable gel into a second chamber, wherein the channel provides fluid communication between the chambers, (c) sealing off the channel, (d) establishing fluid communication between the second chamber and a third chamber of the device, (e) introducing into the third chamber a liquid medium for reagents for amplifying the nucleic acid analyte, when such liquid medium is not already present, (f) transporting the analyte into the third chamber, (g) subjecting the medium to conditions for amplifying nucleic acids, (h) examining the medium for the presence of amplified nucleic acid wherein the order of steps (e) and (f) can be reversed.

The invention further includes kits for conducting an assay for a nucleic acid analyte. The kits comprise in packaged combination a device as described above. The kits can further comprise in packaged combination particles capable of binding the nucleic acid analyte or reagents for amplification or detection of nucleic acids if not present in the device.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a plan view of a device in accordance with the present invention.

Fig. 2 is a plan view of the device of Fig. 1 with its top portion folded on itself.

Fig. 3 is a plan view of another device in accordance with the present invention.

Fig. 4 is a cross-sectional view of the device of Fig. 3.

Fig. 5 is a plan view of another device in accordance with the present invention.

Fig. 6 is a plan view of another device in accordance with the present invention.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

5 As mentioned above, the present invention provides devices and methods for conducting nucleic acid amplification reactions. The present invention is particularly useful for the homogeneous assay of nucleic acids used in conjunction with an amplification procedure.

10 The present devices differ from known devices by, inter alia, the presence of a liquefiable gel or an air gap for transporting the sample bound to particles within a portion of the device. The present device can be utilized to conduct homogeneous nucleic acid assays, that is, assays not requiring a separation step. A further distinction is that some of the present devices have a tandem arrangement of channels and chambers.
15 Finally, the present devices do not include means for separating assay components in a detection chamber as required in some of the prior art devices.

Before proceeding further with a description of the specific embodiments of the present invention, a number of terms will be defined.

20 Nucleic acid or polynucleotide analyte -- a compound or composition to be measured that is a polymeric nucleotide or a portion of a polymeric nucleotide, which in the intact natural state can have about 200 to 500,000 or more nucleotides and in an isolated state can have about 30 to 50,000 or more nucleotides, usually about 100 to 20,000 nucleotides, more frequently
25 500 to 10,000 nucleotides. It is thus obvious that isolation of the analyte from the natural state often results in fragmentation of the polymeric nucleotide. The polynucleotide analytes include nucleic acids from any source in purified or unpurified form including DNA (dsDNA and ssDNA) and RNA, including t-RNA, m-RNA, r-RNA, mitochondrial DNA and RNA,
30 chloroplast DNA and RNA, DNA-RNA hybrids, or mixtures thereof, genes, chromosomes, plasmids, the genomes of biological material such as microorganisms, e.g., bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, humans, and fragments thereof, and the like. The polynucleotide analyte can be only a minor fraction of a complex mixture
35 such as a biological sample. The analyte can be obtained from various biological materials by procedures well-known in the art. Some examples of such biological material by way of illustration and not limitation are disclosed in Table I of U.S. Patent Application Ser. No. 07/923,079 filed July 31, 1992, which Table I is incorporated herein by reference.

40 The polynucleotide analyte, where appropriate, may be treated to cleave the analyte to obtain a polynucleotide fragment that contains a target polynucleotide sequence. Such cleaving treatments may be accomplished, for example, by shearing or by treatment with a restriction endonuclease or other site specific chemical cleavage method. However, it
45 is an advantage of the present invention that the polynucleotide analyte

can be used in its isolated state without further cleavage. The cleaved and uncleaved polynucleotide fragments may each be referred to herein as a polynucleotide analyte.

For purposes of this invention, the polynucleotide analyte, or a cleaved fragment obtained from the polynucleotide analyte, may be at least partially denatured or single stranded or treated to render it denatured or single stranded. Such treatments are well-known in the art and include, for instance, heat or alkali treatment. For example, double stranded DNA, when heated at 90-100° C. for a period of 10-20 seconds or more, produces denatured material.

Sample -- the material suspected of containing the nucleic acid analyte. Such samples include biological fluids such as blood, serum, plasma, sputum, lymphatic fluid, semen, vaginal mucus, feces, urine, spinal fluid, and the like; biological tissue such as hair and skin; and so forth. Other samples include cell cultures and the like, plants, food, forensic samples such as paper, fabrics and scrapings, water, sewage, medicinals, etc.. The sample may be pretreated with reagents to liquefy the sample and release the nucleic acids from binding substances. Such pretreatments are well-known in the art.

Polynucleotide primer -- a polynucleotide, usually a synthetic polynucleotide, usually single stranded and selected in view of the known sequence of the polynucleotide analyte depending on the type of amplification to be conducted. The polynucleotide primer(s) are usually comprised of a sequence of at least 10 nucleotides, preferably, 20 to 90 nucleotides, more preferably, 24 to 64 nucleotides.

Various well-known techniques can be employed for preparing polynucleotide primers. Such sequences can be obtained by biological synthesis or by chemical synthesis. For short sequences (up to about 100 nucleotides) chemical synthesis is frequently more economical as compared to biological synthesis. For longer sequences standard replication methods employed in molecular biology can be used such as the use of M13 for single stranded DNA as described by J. Messing, *Methods Enzymol*(1983) 101: 20-78.

In addition to standard cloning techniques, in vitro enzymatic methods may be used such as polymerase catalyzed reactions. For preparation of RNA, T7 RNA polymerase and a suitable DNA template can be used. For DNA, polymerase chain reaction (PCR) and single primer amplification are convenient.

Other chemical methods of polynucleotide or oligonucleotide synthesis include phosphotriester and phosphodiester methods (Narang, et al., *Meth. Enzymol* (1979) 68: 90) and synthesis on a support (Beaucage, et al., *Tetrahedron* (1981) *Letters* 22: 1859-1862) as well as phosphoramidate technique, Caruthers, M. H., et al., "Methods in Enzymology," Vol. 154, pp. 287-314 (1988), and others described in "Synthesis and Applications of DNA and RNA," S.A. Narang, editor, Academic Press, New York, 1987, and the references contained therein.

Deoxynucleoside triphosphates -- deoxynucleosides having a 5'-triphosphate substituent. The deoxynucleosides are pentose sugar derivatives of nitrogenous bases of either purine or pyrimidine derivation, covalently bonded to the 1'-carbon of the pentose sugar. The purine bases include adenine(A), guanine(G), inosine, and derivatives and analogs thereof. The pyrimidine bases include cytosine (C), thymine (T), uracil (U), and derivatives and analogs thereof.

The derivatives and analogs include any substrate of a polydeoxynucleotide polymerase that can be incorporated into a polynucleotide through catalysis by such enzyme. The derivatives and analogs are exemplified by those that are recognized and polymerized by the enzyme in a similar manner to the underivitized nucleoside triphosphates. Examples of such derivatives or analogs by way of illustration and not limitation are those that are modified with a reporter group, biotinylated, amine modified, radiolabeled, alkylated, and the like and also include phosphorothioate, phosphite, ring atom modified derivatives, unnatural bases, and the like. The reporter group can be a fluorescent group such as fluorescein, a chemiluminescent group such as luminol, a terbium chelator such as N-(hydroxyethyl) ethylenediaminetriacetic acid that is capable of detection by delayed fluorescence, and the like.

Amplification of nucleic acids or polynucleotides -- any method that results in the formation of one or more copies of a nucleic acid or polynucleotide molecule, usually a nucleic acid or polynucleotide analyte, present in a medium. One such method for the enzymatic amplification of specific double stranded sequences of DNA is known as the polymerase chain reaction (PCR), as described above. This in vitro amplification procedure is based on repeated cycles of denaturation, oligonucleotide primer annealing, and primer extension by thermophilic template dependent polynucleotide polymerase, resulting in the exponential increase in copies of the desired sequence of the polynucleotide analyte flanked by the primers. The two different PCR primers, which anneal to opposite strands of the DNA, are positioned so that the polymerase catalyzed extension product of one primer can serve as a template strand for the other, leading to the accumulation of a discrete double stranded fragment whose length is defined by the distance between the 5' ends of the oligonucleotide primers.

Another method for amplification is mentioned above and involves amplification of a single stranded polynucleotide using a single polynucleotide primer. The single stranded polynucleotide that is to be amplified contains two non-contiguous sequences that are complementary to one another and, thus, are capable of hybridizing together to form a stem-loop structure. This single stranded polynucleotide may be already part of a polynucleotide analyte or may be created as the result of the presence of a polynucleotide.

Another method for achieving the result of an amplification of nucleic acids is known as the ligase chain reaction (LCR). This method

uses a ligase enzyme to join preformed nucleic acid probes. The probes hybridize with the nucleic acid analyte, if present, and ligase is employed to link the probes together resulting in two templates that can serve in the next cycle to reiterate the particular nucleic acid sequence.

5 Another method for achieving a nucleic acid amplification is the nucleic acid sequence based amplification (NASBA). This method is primer-directed, enzymatic process that induces in vitro continuous, homogeneous and isothermal amplification of specific nucleic acid.

10 Another method for amplifying a specific group of nucleic acids is the Q-beta-replicase method, which relies on the ability of Q-beta-replicase to amplify its RNA substrate exponentially.

Polydeoxynucleotide polymerase -- a catalyst, usually an enzyme, for forming an extension of the polynucleotide primer along a nucleic acid template that is comprised predominantly of deoxynucleotides. The
15 polydeoxynucleotide polymerase is a template dependent polydeoxynucleotide polymerase and utilizes the deoxynucleoside triphosphates as building blocks for extending the 3' end of the polynucleotide primer to provide a sequence complementary with a single stranded polynucleotide sequence. Usually, the catalysts are enzymes, such as DNA polymerases, for example,
20 prokaryotic DNA polymerase (I, II, or III), T4 DNA polymerase, T7 DNA polymerase, Klenow fragment, reverse transcriptase, Vent DNA polymerase (Vent is a trademark of New England BioLabs, Beverly, MA), Pfu DNA polymerase, Taq DNA polymerase, and the like, derived from any source such as cells, bacteria, for example, E. coli, plants, animals, virus,
25 thermophilic bacteria, and so forth. Where the target polynucleotide sequence is RNA, reverse transcriptase is used as at least one of the polynucleotide polymerases to facilitate extension of the primer along the complementary strands of the polynucleotide analyte.

Hybridization (hybridizing) and binding -- in the context of
30 nucleotide sequences these terms are used interchangeably herein. The ability of two polynucleotide sequences to hybridize with each other is based in a large part on the degree of complementarity of the two polynucleotide sequences, which in turn is based on the fraction of matched complementary nucleotide pairs. The more nucleotides in a given sequence
35 that are complementary to another sequence, the more stringent the conditions can be for hybridization and the more specific will be the binding of the two sequences. Another factor to be considered is the nature of the nucleotide pairs that are opposite in the two strands. Some nucleotide pairs, such as G and C, have greater binding affinities for one
40 another than do other pairs. Increased stringency is achieved by elevating the temperature, increasing the ratio of cosolvents, lowering the salt concentration, and the like.

Homologous or substantially identical -- In general, two
45 polynucleotide sequences that are identical, or at least can each hybridize to the same polynucleotide sequence, are homologous. The two sequences are

-10-

homologous or substantially identical where the sequences each have at least 90%, preferably 100%, of the same or analogous base sequence where thymine (T) and uracil (U) are considered the same. Thus, the ribonucleotides A, U, C and G are taken as analogous to the deoxynucleotides dA, dT, dC, and dG, respectively. Homologous sequences can both be DNA or one can be DNA and the other RNA.

Complementary--two sequences are complementary when the sequence of one can bind to the sequence of the other in an anti-parallel sense wherein the 3' end of each sequence binds to the 5' end of the other sequence and, for example, among the natural bases each A, T(U), G, and C of one sequence is then aligned with a T(U), A, C, and G, respectively, of the other sequence.

Copy -- a sequence that is identical to or homologous with a single stranded polynucleotide sequence as differentiated from a sequence that is complementary to or hybridizable with the sequence of such single stranded polynucleotide.

Member of a specific binding pair ("sbp member") -- one of two different molecules, having an area on the surface or in a cavity that specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of the other molecule. The members of the specific binding pair are referred to as ligand and receptor (antiligand). These may be members of an immunological pair such as antigen-antibody, or may be operator-repressor, nuclease-nucleotide, biotin-avidin, hormones-hormone receptors, nucleic acid duplexes, IgG-protein A, DNA-DNA, DNA-RNA, and the like.

Ligand -- any compound for which a receptor naturally exists or can be prepared.

Receptor ("antiligand") -- any compound or composition capable of recognizing a particular spatial and polar organization of a molecule, e.g., epitopic or determinant site. Illustrative receptors include naturally occurring receptors, e.g., thyroxine binding globulin, antibodies, enzymes, Fab fragments, lectins, nucleic acids, repressors, protection enzymes, protein A, complement component C1q, DNA binding proteins or ligands and the like.

Small organic molecule -- a compound of molecular weight less than 1500, preferably 100 to 1000, more preferably 300 to 600 such as biotin, fluorescein, rhodamine and other dyes, tetracycline and other protein binding molecules, and haptens, etc. The small organic molecule can provide a means for attachment of a nucleotide sequence to a label or to a particle.

Particles -- non-magnetic or magnetic beads of approximately 50 to 10000 nanometers, preferably 300 to 5000 nanometers. The non-magnetic particles are usually diamagnetic or paramagnetic with a magnetic susceptibility (χ) of less than 1×10^{-5} emu/g Oe cm³. The non-magnetic particles may be organic or inorganic, swellable or non-swellable, porous

or non-porous, usually of a density heavier than water, generally about 1.1 to 1.3, preferably 2 to 10 g/ml. The particles can be, for example, organic or inorganic polymers, latex particles, inorganic powders such as silica, magnesium sulfate, and alumina; natural polymeric materials, synthetic or modified naturally occurring polymers, such as nitrocellulose, cellulose acetate, poly (vinyl chloride), polyacrylamide, cross linked dextran, agarose, polyacrylate, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), nylon, poly(vinyl butyrate), etc.; either used by themselves or in conjunction with other materials; glass available as Bioglass, ceramics, metals, and the like.

The magnetic particles are intrinsically magnetically responsive or have been rendered magnetic by, for example, attachment to a magnetically responsive substance or by incorporation of such substance into the particles. The magnetic particles can be paramagnetic, ferromagnetic, or superparamagnetic, usually paramagnetic or superparamagnetic and have magnetic susceptibilities (χ) of at least 5×10^{-5} , usually 4×10^{-4} , emu/g. Exemplary of the magnetic component of particles that are intrinsically magnetic or magnetically responsive are complex salts and oxides, borides and sulfides of iron, cobalt, nickel and rare earth elements having high magnetic susceptibility, e.g., hematite, ferrite and so forth. The magnetic component of other such particles includes pure metals or alloys comprising one or more of these elements.

Binding of sbp members to particles may be accomplished by well-known techniques, commonly available in the literature. See, for example, "Immobilized Enzymes," Ichiro Chibata, Halsted Press, New York (1978) and Cuatrecasas, *J. Biol. Chem.*, 245:3059 (1970).

Label or reporter group or reporter molecule -- a member of the signal producing system. Usually the label or reporter group or molecule is conjugated to or becomes bound to a polynucleotide probe or a polynucleotide primer and is capable of being detected directly, or indirectly by being bound through a specific binding reaction, to a detectable substance. Labels able to be detected indirectly include polynucleotides such as a polynucleotide primer or a specific polynucleotide sequence that can act as a ligand for a complementary polynucleotide or provide a template for amplification or ligation or act as a ligand such as for a repressor protein; haptens; antibodies; receptors such as avidin; ligands such as biotin and the like. Labels able to be detected directly may be isotopic or nonisotopic, usually non-isotopic, and can be a catalyst, such as an enzyme, ribozyme, a substrate for a replicase such as QB replicase, promoter, dye, fluorescent molecule, chemiluminescer, coenzyme, enzyme substrate, radioactive group, a particle such as latex or carbon particle, metal sol, crystallite, liposome, cell, etc., which may or may not be further labeled with a dye, catalyst or other detectable group, and the like. The label is a member of a signal producing system and can

-12-

generate a detectable signal either alone or together with other members of the signal producing system. The label can be bound directly to a nucleotide sequence or can become bound thereto by being bound to an sbp member complementary to an sbp member that is bound to a nucleotide sequence. Methods for binding of labels to nucleotides are well-known and described, for example, in U.S. Patent No. 4,948,882 (Ruth), U.S. Patent No. 5,082,830 (Brakel, et al.), U.S. Patent No. 4,894,325 (Koser, et al.) and U.S. Patent No. 4,987,065 (Stavrianopolis, et al.).

Signal Producing System -- The signal producing system may have one or more components, at least one component being the label or reporter group. The signal producing system generates a signal that relates to the presence or amount of nucleic acid analyte in a sample. The signal producing system includes all of the reagents required to produce a measurable signal. When the label is not conjugated to a nucleotide sequence, the label is normally bound to an sbp member complementary to an sbp member that is bound to or part of a nucleotide sequence. Other components of the signal producing system may be included in a developer solution and can include substrates, enhancers, activators, chemiluminescent compounds, cofactors, inhibitors, scavengers, metal ions, specific binding substances required for binding of signal generating substances, and the like. Other components of the signal producing system may be coenzymes, substances that react with enzymic products, other enzymes and catalysts, and the like. The signal producing system provides a signal detectable by external means, such as detection of electromagnetic radiation, desirably by visual examination. The signal-producing system is described more fully in U.S. Patent Application Serial No. 07/555,323, filed July 19, 1990, the relevant disclosure of which is incorporated herein by reference.

Ancillary Materials -- Various ancillary materials will frequently be employed in the method in accordance with the present invention. For example, buffers will normally be present in the medium, as well as stabilizers for the medium and the reaction components. Frequently, in addition to these additives, proteins may be included, such as albumins, organic solvents such as formamide, quaternary ammonium salts, polyanions such as dextran sulfate, surfactants, particularly non-ionic surfactants, binding enhancers, e.g., polyalkylene glycols, or the like.

One such device in accordance with the present invention is depicted in Figs. 1 and 2. Device 10 has a sample receiving channel 12 with port 14 for introduction of sample into device 10. Channel 12 is in fluid communication with first chamber 16 and with second channel 18, which is in potential fluid communication with second chamber 20. The fluid communication between channel 18 and chamber 20 is temporarily prevented by temporary seal 22, thus rendering the fluid communication between channel 18 and chamber 20 potential. Both channel 18 and chamber 20 are filled with a heat meltable or liquifiable gel 24. Chamber 20 is in potential

-13-

fluid communication with a third channel 26, having a temporary seal 27. Channel 26 is in fluid communication with third chamber 28.

Device 10 is constructed such that top portion 30 is foldable on itself along line 32, thus permitting channel 12 to be sealed off from the environment as seen in Fig. 2. This is by way of example and not limitation. Sealing of the device after sample has been introduced through port 14 may be accomplished by other means such as heat sealing of the port or of channel 12 at a point adjacent to port 14. Alternatively, port 14 may be closed by pinching, stoppering, capping and the like. Port 14 may also comprise a self-sealing elastomer.

The liquefiable or heat meltable gel is a thermally liquefiable gel that may be a polysaccharide, polyacrylate, polypeptide, polyvinylalcohol, polyether, polysiloxane or the like. The thermally liquefiable gel is liquefiable at a temperature of about 35 to 105° C, preferably, 45 to 95° C, more preferably, 60 to 70° C. It is important to note that with some gels it is not necessary that the entire gel be liquified to achieve the necessary movement of particles with the nucleic acid analyte bound thereto through the gel. In general, the thermally liquefiable gel, when liquified, has a viscosity that permits movement of the nucleic acid bound to particles through the gel; usually a viscosity of about 0.005 to 0.5 poise, preferably 0.01 to 0.05 poise. The mass density of the gel may be adjusted by adding to the gel a heavy metal salt, such as cesium chloride, or polyiodinated aromatic compounds such as those sold under the trade name NYCODENZ, in an amount sufficient to achieve the desired density, preferably 1.05 to 1.2 g/cm³. In general, the mass density of the gel is greater than that of the sample to ensure that the sample and the liquified gel are stably stratified, thereby avoiding mixing and subsequent contamination of the remainder of the device with the sample. In general, the density or viscosity of the gel is greater than that of the sample to prevent the liquified gel and the sample from mixing, thereby avoiding contamination of the remainder of the device with the sample.

Materials useful as the thermally liquefiable gel, by way of example and not limitation, are the following: polysaccharides such as agarose; polyacrylates such as polyacrylamide; polypeptides such as gelatin; polyvinylalcohols; polyethers; and polysiloxanes. A preferred gel to adjust the density is agarose (0.5% w/v) (e.g., SeaPlaque®, FMC, Bioproducts, Rockland, ME with a polyiodinated aromatic compound such as Nycodenz® which is a non-ionic tri-iodinated derivative of benzoic acid with three aliphatic hydrophilic side chains. The systematic name of Nycodenz® is 5-(n-2,3-dihydroxypropylacetamido)-2,4,6-tri-iodo-n, n'-bis(2,3 dihydroxypropyl) isophthalamide. (Nycodenz®, Nycomed AS, Oslo, Norway). In one embodiment of the invention the liquefiable gel serves as the only solvent for the sample.

The appropriate channel and chamber of the device can be filled with gel during manufacture of the device by injecting molten gel into the

-14-

channel or chamber or both. Air pocket formation is preferably avoided. During this procedure the device may be supported vertically on a rigid backing to prevent the gel from being disrupted as the device is handled during the procedure. The gel is then cooled to facilitate solidification.

5 The breaking of a frangible seal is one means for permitting the establishment of fluid communication between a channel and a chamber or between chambers. Other means for permitting the establishment of fluid communication includes relieving the pressure on a seal produced by pressure on the flexible wall of a channel, applying hydrostatic pressure
10 to a channel sealed by surface tension, charge, and/or Van der Waals forces, unbinding a channel that is sealed as a result of a bend, melting a wax, crystalline, or gel plug in a channel, photodepolymerization of a polymer comprising a gel, etc.

15 Fluid communication between a channel and a chamber or between chambers can be terminated by means for sealing off the fluid communication. Such means can be, for example, a seal produced by application of external pressure, a portion of the device foldable on itself as described above for the port, heatsealing, freezing, photopolymerization of a monomer, and so forth.

20 In general, devices in accordance with the present invention are preferably composed of at least one flexible layer and a second layer that may be flexible or rigid. For example, the device can be manufactured from two sheets of flexible plastic sealed together and having interior blisters or bubbles forming the chambers and channels. Preferably, the first
25 chamber is larger than the other chambers, but need not be. The volume of the sample receiving or first chamber is usually about 0.02 to 2 ml, preferably, 0.1 to 1.0 ml. The volume of each of the remaining chambers is usually independently about 0.02 to 1 ml, preferably, 0.1 to .5 ml. The volume of the liquefiable gel is usually about 0.1 to 3 ml, preferably, 0.2
30 to 2 ml. Generally, this volume of gel is distributed between the first channel and the second chamber, both of which contain the gel.

The material for use in manufacturing a device in accordance with the present invention should be thermally stable, provide a moisture barrier, be chemically compatible with the reagents used for the handling and
35 transfer of sample and amplification and detection of amplified nucleic acid and compatible with the various procedures such as heatsealing employed in using the device, and be sealable and formable.

The material for fabrication of the present device should be flexible enough to permit the device to be manipulated during liquid transfer steps
40 involving squeezing of parts of the device to force contents from one area of the device to another. The material should not soften appreciably at temperatures up to about 100° C. Moisture barrier properties of the material can be quantified by the moisture vapor transmission rate (MVTR) measured in g-mil/100 sq. in. /24 hour. Preferred MVTR at room temperature
45 for the material is less than about 0.2, preferably less than, 0.05 g-

-15-

mil/100 sq in /24 hour. Use of lamination of two or more different sheets of plastic film helps to reduce the MVTR values.

Generally, the thickness of the plastic film or sheets used in the manufacture of a device in accordance with the present invention is about 1 to 6 mil, preferably, 2 to 4 mil. These values apply to the plastic film as a single material or as a laminate.

Suitable plastics that may be used in the present device are, by way of example and not limitation, polyolefins such as polypropylene and low or medium density polyethylene; ethylene-vinyl acetate; polyvinylidene chloride; chlorotrifluoroethane; and the like and laminations of two or more of the above. It is also within the scope of the present invention to use laminates of one or more of the above plastics with a plastic other than that recited specifically above as long as the requisite characteristics of the device are obtained.

Frequently, the device is comprised of two plastic films, usually heat sealed together. The device may further be mounted on a rigid backing, usually plastic such as polyvinylchloride (PVC), polycarbonate, nylon, polyethylene, etc., or glass or metal. Alternatively, one plastic film can be sealed directly to a rigid backing, which then usually is a plastic material.

In an initial step in the manufacture of the present device one sheet of plastic used to form the device is formed into bubbles or blisters to make the various channels and chambers of the device. This is usually accomplished by stretching the sheet or film of plastic over or into a mold. Thermoforming is the most common method of accomplishing this molding. The film is placed on a forming die that has been cut to create the shapes of the blisters. Vacuum and heat are applied during the thermoforming process. The plastic used in this sheet must flow and thin so that it can be formed into the appropriate shapes. Laminates are particularly suitable for this purpose.

Once the film is formed into the desired shape, it is joined with another film or sheet of plastic or a backing, which may or may not be of the same composition as the first film. Numerous methods are available to join the plastic films together. One such method is heatsealing using a hot die. Many apparatus for heatsealing are commercially available. Other methods that are variations of heatsealing include ultrasonic welding, RF sealing and vibration sealing. Heatsealing is often carried out using a laminate with a specific heat seal layer on the side to be sealed, typically polyethylene or ethylene vinyl acetate. Other methods for joining the plastic films are solvent bonding and adhesive bonding.

The overall dimensions of the device are a matter of choice for the particular application of the device. In general, the device is about 2 to 25 cm, preferably, 4 to 15 cm, in length, about 8 to 30 cm, preferably, 10 to 20 mm, in width, and about 0.1 to 4 mm, preferably, 2 to 2 mm, in depth.

The material used to make the dies for the forming and heat sealing

-16-

steps should preferably allow low heat flux into the die, thereby decreasing the gradient across the plastic films. Heat seal temperature can then be reached at lower platen temperatures. The material preferably should be easy to machine and able to hold its shape after many applications of heat and pressure. Desirably, the material should be slightly compressible under the platen pressures employed in the forming and heatsealing steps. Suitable materials, by way of example and not limitation, are printmaking linoleum, e.g., such as manufactured by Speedball Printmaster® (Hunt Mfg. Co., Philadelphia, PA) metal such as aluminum; silicone rubber; gasket paper; phenolic composite; laboratory labeling tape; and the like.

The heat sealing step is conducted at a platen temperature of about 260 to 320, preferably, 280 to 300°F. with a contact time of about 0.2 to 5, preferably, 0.5 to 2 seconds and at a platen pressure of about 200 to 1200, preferably, 600 to 900 pounds per square inch (psi). For the formation of a weak frangible seal in a channel or chamber of the present device, the heat sealing conditions should be balanced to achieve both a weak frangible seal and a strong seal over the perimeter of the device. The frangible seal is formed in a groove that is cut shallow in the die. Because of poor thermal contact, the interface in this groove heats up more slowly than the perimeter seal. For a linoleum die and a plastic laminate of 0.75 to 2.0 mil polychlorotrifluoroethane/polyethylene the platen temperature is usually about 290 to 300°C. with a platen pressure of about 600 to 800 psi and a contact time of about 0.7 to 0.9 seconds. To achieve a sufficient frangible seal with this laminate the platen is lifted before the groove seal reaches melt strength. In general, the frangible seal in the present device should break with an applied force of about 5 to 15 pounds. A balance must be achieved between breaking the frangible seal and breaking the perimeter seal of the device.

Another method to achieve the appropriate frangible and perimeter seals involves interposing at the point of the frangible seal a substance that interferes with the heatseal. This results in a seal at that point that is weaker than the perimeter seal. Upon application of pressure to the channel or chamber containing the frangible seal, the seal breaks more readily than the perimeter seal. Such substances are generally oily or tacky and may be, by way of example and not limitation, a solvent-based adhesive that retains some elasticity upon evaporation of the solvent such as a mixture of transfer tape adhesive (e.g., 3M Company) and silicone fluid dissolved in xylene and thinned with methylene chloride (1 part adhesive, 1 part silicone fluid, 2 parts xylene and 2 parts methylene chloride), rubber cement thinned in methylene chloride (1 part rubber cement and 1 part methylene chloride), a substance contained in the PAP Pen (Daido Sangyo, Japan), and the like.

A blister pack device in accordance with the present invention is depicted in Figs. 3 and 4. Device 40 has entry port 42 for introduction of

-17-

sample into device 40. Port 42 is in fluid communication with sample receiving chamber 44. The bottom portion 46 of chamber 44, channel 48 and chamber 50 are in fluid communication and are filled with a heat meltable or liquifiable gel 52. Neck 51 is capable of being sealed off by application of pressure. Frangible seal 54 in channel 56 prevents fluid communication between chamber 50 and chamber 58. Chamber 50 may contain one or more reagents for conducting an amplification of nucleic acids and chamber 58 may contain one or more reagents for the detection of amplified nucleic acids.

Another embodiment of the present invention is a method for amplifying and detecting a nucleic acid analyte using a sealable device. The method comprises the steps of: (a) introducing a sample suspected of containing the nucleic acid analyte into a first chamber of the device preceding or following combining the sample with particles capable of binding the nucleic acid analyte, (b) transporting the particles from the first chamber through a channel containing air or a liquefiable gel into a second chamber, wherein the channel provides fluid communication between the chambers, (c) sealing off the channel, (d) establishing fluid communication between the second chamber and a third chamber of the device, (e) introducing into the third chamber a liquid medium for reagents for amplifying the nucleic acid analyte, when such liquid medium is not already present, (f) transporting the analyte into the third chamber, (g) subjecting the medium to conditions for amplifying nucleic acids, (h) examining the medium for the presence of amplified nucleic acid wherein the order of steps (e) and (f) can be reversed.

The method is next described in more detail with reference to the devices of the present invention depicted in Figs. 5 and 6. In a first step the sample is combined with particles capable of binding the nucleic acid analyte. Alternatively, particles capable of binding the nucleic acid analyte can be present in the first chamber 72 of device 70. In either event the sample is introduced into the device through port 74 and top portion 94 is folded along line 92 to seal the port.

The particles are capable of binding the nucleic acid analyte by virtue of having bound thereto a polynucleotide sequence capable of hybridizing to, and preferably complementary to, the nucleic acid analyte. The particles can be non-magnetic or magnetic, preferably magnetic.

In one embodiment of the invention the particles are non-magnetic with a density heavier than water to permit separation from the medium containing the sample through settling and movement of the particles from the first chamber through channel 76 to the second chamber 78 after breaking of frangible seal 79. If a gel is employed, the particles should have a density greater than that of the gel. The gel is in lower portion 80 of chamber 72 and in channel 76 of device 70.

Alternatively, the sample can be combined with magnetic particles capable of binding the nucleic acid analyte and moved through the sample

-18-

medium or air, or through liquefied gel if a gel is employed, by application of a magnetic field gradient such as by magnet 82 in Fig. 5. For magnetic transfer through a gel it is first necessary to partially or fully liquefy the gel. Usually, this is accomplished by heating the channel or chamber containing the gel to an appropriate temperature. For partially liquefied gel, the magnetic particle mass is transferred through the area of liquefaction. The gel in at least this area should be at a viscosity that permits the particles to move.

It is also within the scope of the present invention to transfer the magnetic particle mass through an air pocket 108 in the device 100 of Fig. 6, for example, in the channel 106 leading from the first chamber 104 to the second chamber 78. Medium containing sample and magnetic particles is introduced into device 100 through port 102. Top portion 122 is folded on itself along line 120 to seal off the port. In this approach the medium 114 containing the magnetic particle mass is subjected to a magnetic field gradient produced by magnet 118 to pull the magnetic particle mass out of the medium and into air pocket 108, which is usually an unwetted area 116 of the plastic film forming the chambers and/or channels of the device through which the particle mass must move. In some cases the magnetic particles adhere to the inner plastic surface of the channel and/or chamber. One way of reducing or eliminating this adherence is to coat the inner surface with a detergent such as, for example, Tween 20[®] (Triton X100[®], lecithin) or the like. However, a balance must be reached between elimination of adherence and increasing the wetting capability of the surface by the detergent. Generally, a relatively strong magnetic field gradient is necessary to move the magnetic particle mass along the plastic surface in this embodiment. Such a gradient should be about 5 to 15 KOe/cm and may require that the magnet be in contact with the plastic film as the particles move along. More than one magnet may be used to achieve a gradient sufficient to move the magnetic particle mass, which should be as compact as possible. The magnet, and thus the magnetic particle mass, usually are moved at a speed of about 0.5 to 20 mm/sec.

Once the particles have been transferred to second chamber 78 (Fig. 5 and 6, respectively), channel 76 between the first and second chambers is sealed by one of the methods described above. The contents of chamber 78 can be mixed by forcing the contents back and forth into and out of channel 76 or 108. The second chamber can contain reagents 88 for conducting amplification of the nucleic acid analyte if present. These reagents can be present in a liquid or, preferably, dry, form such as a tablet or powder and can include template dependent polynucleotide polymerase, deoxynucleotide triphosphates, and polydeoxynucleotide primer(s). The amplification can be conducted in this second chamber 78 or the contents of the second chamber can be transferred to the third chamber 84, where the amplification can be carried out. Fluid communication is established between second chamber 78 and a third chamber 84 usually by breaking a

-19-

frangible seal 86, which can be broken by applying pressure to the contents of chamber 78 and forcing the contents into chamber 84. The third chamber can contain reagents 90 for detecting amplified nucleic acid or such reagents can be introduced into the second or third chamber after amplification has been carried out. This may be accomplished by forcing a liquid containing these reagents through a channel (not shown) in fluid communication with chamber 84 wherein the fluid can be contained in a separate chamber in fluid communication with the channel and the channel may optionally be sealed with a frangible seal. Fourth chamber 96 is in potential fluid communication with third chamber 90. At an appropriate time fluid communication is established between chambers 96 and 90 and the reaction mixture passes into chamber 96 where, for example, signal is read.

Appropriate reaction conditions are chosen for carrying out the amplification reaction. The following description sets forth such appropriate conditions, which are subject to modification by those skilled in the art depending on the specific reagents and other molecules chosen for any particular application.

Generally, an aqueous medium is employed. Other polar cosolvents may also be employed in the medium, usually oxygenated organic solvents of from 1-6, more usually from 1-4, carbon atoms, including alcohols, ethers and the like. Usually, these cosolvents are present in less than about 70 weight percent, more usually, in less than about 30 weight percent.

The pH for the medium is usually in the range of about 5.5 to 10, more usually, in the range of about 6.5 to 9.5, and, preferably, in the range of about 7 to 9. The pH and temperature are chosen and varied, as the case may be, so as to cause, either simultaneously or wholly or partially sequentially, dissociation of any internally hybridized sequences, hybridization of the primer with the single stranded polynucleotide sequences and extended primer once the primer has been extended, extension of the primer along the single stranded polynucleotide sequences and extended primer, and dissociation of the extended primer from its duplex. In some instances, a compromise will be made in optimizing the speed, efficiency, and specificity of these steps depending on whether it is desired to perform the above steps wholly or partially sequentially or simultaneously. Various buffers may be used to achieve the desired pH and maintain the pH during the determination. Illustrative buffers include borate, phosphate, carbonate, Tris, barbital and the like. The particular buffer employed is not critical to this invention but in individual methods one buffer may be preferred over another.

Moderate temperatures are normally employed for carrying out the amplification. The temperature employed are dependent on a number of considerations such as, for example, the salt concentration and the pH of the medium, the solvent composition of the medium used, the length of the polynucleotide analyte and the length and nucleotide composition of the primer(s).

-20-

Normally, in conducting the method of the invention for amplification of nucleic acids, the medium is cycled between two or three temperatures. To this end the device in accordance with the present invention is placed in a liquid, usually water, bath and the temperature of the bath is varied accordingly or the chamber containing the combined analyte and amplification reagent is placed in contact with a thermally conductive surface, the temperature of which is varied. The temperatures for the present method in conjunction with amplification generally range from about 10° to 105°C, more usually from about 40° to 99°C, preferably 50° to 98°C. Relatively low temperatures of from about 30° to 75°C can be employed for the hybridization steps, while denaturation and extension can be carried out at a temperature of from about 50° to 105°C. The amplification is conducted for a time sufficient to achieve a desired number of copies to achieve an accurate assay for a polynucleotide analyte. Generally, the time period for conducting the method is from about 20 seconds to 10 minutes per cycle and any number of cycles can be used from 1 to as high as 100 or more, usually 5 to 80, frequently 10-60. As a matter of convenience it is usually desirable to minimize the time period and the number of cycles. In general, the time period for a given degree of amplification can be shortened, for example, by selecting concentrations of nucleoside triphosphates sufficient to saturate the polynucleotide polymerase and by increasing the concentrations of polynucleotide polymerase and polynucleotide primer. Generally, the time period for conducting the method is from about 5 to 200 minutes.

Amplified nucleic acid can be detected in numerous ways. For example, molecules of the polynucleotide primer can be labeled with a reporter molecule such as a ligand, a small organic molecule including fluorescers, chemiluminescers and the like, catalysts, co-enzymes, radioactive substances, amplifiable polynucleotide sequences, a polypeptide, a support, an operator or the like. Examples of particular labels or reporter molecules and their detection can be found in U.S. Patent Application Serial No. 07/555,323 filed July 19, 1990, the relevant disclosure of which is incorporated herein by reference. Other assay formats and detection formats are disclosed in U.S. Patent Applications Serial Nos. 07/229,282 and 07/399,795 filed January 19, 1989, and August 29, 1989, respectively, U.S. Patent Application Serial No. 07/555,323 filed July 19, 1990, U.S. Patent Application Serial No. 07/555,968 filed July 19, 1990, U.S. Patent Application Serial No. 07/776,538 filed October 11, 1991, U.S. Patent Application Serial No. 07/923,079 filed July 31, 1992, all of which have been incorporated herein by reference. Any standard method for specifically detecting nucleic acid sequences can be used.

One particular method for detecting amplified nucleic acid is described in U.S. Patent Application Ser. No. 07/704,569 filed May 22, 1991 (Ullman, et al.), the relevant disclosure of which is incorporated herein by reference. The method comprises treating the reaction mixture suspected

SUBSTITUTE SHEET (RULE 26)

-21-

of containing the amplified nucleic acid analyte under conditions such that the analyte, if present, causes a photosensitizer and a chemiluminescent compound to come into close proximity. The photosensitizer generates singlet oxygen and activates the chemiluminescent compound when it is in close proximity. The activated chemiluminescent compound subsequently produces light. The amount of light produced is related to the amount of analyte in the medium. More particularly, as applied to the present invention, the method comprises as a first step providing a combination comprising the aforementioned medium suspected of containing amplified nucleic acid analyte bound to a particle which also has a chemiluminescent compound associated with the particle, a photosensitizer associated with a specific binding pair (sbp) member capable of binding to the amplified nucleic acid analyte. The combination is treated, usually by irradiation with light, to excite the photosensitizer, which is capable in its excited state of activating oxygen to a singlet state. The combination is then examined for the amount of luminescence or light emitted. The amount of such luminescence is related to the amount of nucleic acid analyte in the medium. Alternatively, the chemiluminescent compound is associated with an sbp member capable of binding amplified nucleic acid analyte and the particle to which the nucleic acid analyte is bound is associated with a photosensitizer.

Signal generated by the detection reagents is generally measured or detected at the chamber where the reaction mixture is located. However, it is within the purview of the present invention to transfer the reaction mixture to a fourth chamber prior to reading the signal. For example, in Fig. 5 detection of signal can occur at the third chamber 84. The chamber at which detection of signal occurs usually contains means for detection of the signal such as, for example, an element capable of transmitting a signal, e.g., a window or electrode depending on the nature of the signal formed. In one embodiment of the invention an optical signal is read through the wall of the chamber containing the reaction mixture.

As a matter of convenience, a device in accordance with the present invention can be provided in packaged combination in a kit, which also includes predetermined amounts of reagents employed in the present invention that are not included in the device. The kits can be used in accordance with the methods of the present invention in determining a polynucleotide analyte. In one embodiment the kit comprises in packaged combination: (a) a device in accordance with the present invention and (b) particles capable of binding the nucleic acid analyte.

The kit can further include a labeled or unlabeled polynucleotide probe capable of binding to extended primer produced in the method of the invention. The kits above can further include in the packaged combination, if not included in the device, deoxynucleoside triphosphates (dNTPs) such as, e.g., deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP) and deoxythymidine triphosphate

SUBSTITUTE SHEET (RULE 26)

-22-

(dTTP) or derivatives or analogs of the above. The kit can further include a polydeoxynucleotide polymerase and members of a signal producing system and also various buffered media, some of which may contain one or more of the above reagents.

5 The relative amounts of the various reagents in the kits can be varied widely to provide for concentrations of the reagents that substantially optimize the reactions that need to occur during the present method and to further substantially optimize the sensitivity of any assay, in which the present method is employed. Under appropriate circumstances
10 one or more of the reagents in the kit can be provided as a dry powder, usually lyophilized, including excipients, which on dissolution will provide for a reagent solution having the appropriate concentrations for performing a method or assay in accordance with the present invention. Each reagent can be packaged in separate containers or some reagents can be
15 combined in one container where cross-reactivity and shelf life permit.

 The above discussion includes certain theories as to mechanisms involved in the present invention. These theories should not be construed to limit the present invention in any way, since it has been demonstrated that the present invention achieves the results described.

20 The above description and examples disclose the invention including certain preferred embodiments thereof. Modifications of the methods described that are obvious to those of ordinary skill in the art such as molecular biology and related sciences are intended to be within the scope of the following claims and included within the metes and bounds of the
25 invention.

SUBSTITUTE SHEET (RULE 26)

-23-

What is Claimed is:

1. A device for carrying out an assay for the determination of a nucleic acid, said device comprising:

- 5 (a) a sample receiving chamber,
(b) a plurality of additional chambers, at least one of said additional chambers containing reagents for carrying out an assay for the determination of a nucleic acid,
(c) means for detecting a signal generated by said reagents,
10 (d) means for permitting fluid communication between said additional chambers,
(e) means for introducing a sample into said device, and
(f) means for separating said nucleic acid from the remainder of said sample and introducing said nucleic acid into one of said additional chambers said means comprised of suspendible particles.
- 15

2. A device for amplifying and detecting a nucleic acid analyte, said device comprising:

- 20 (a) a port for introducing a sample suspected of containing said nucleic acid analyte into said device,
(b) a first chamber for receiving said sample in fluid communication with said port, wherein fluid communication between said port and said first chamber is capable of being sealed off,
(c) a channel containing a liquefiable gel, said channel being in
25 fluid communication with said first chamber,
(d) a second chamber containing reagents for amplifying said nucleic acid analyte, fluid communication between said second chamber and said channel being prevented by a temporary seal, and
(e) a third chamber, fluid communication between said second
30 chamber and said third chamber being prevented by a temporary seal.

3. The device of Claim 2 wherein said liquefiable gel is selected from the group consisting of polysaccharides, polyacrylates, polypeptides, polyvinylalcohols, polyethers and polysiloxanes.

35

4. The device of Claim 2 that is a flexible package.

5. A device for carrying out an assay for an analyte in a sample, said device comprising:

- 40 (a) a port for introducing said sample into said device,
(b) a first chamber for receiving said sample,
(c) a channel containing a liquifiable gel, said channel being connected to said first chamber,
(d) a second chamber connected to said channel, and
45 (e) particles in said first chamber that are capable of being

SUBSTITUTE SHEET (RULE 26)

-24-

transported through said liquifiable gel into said second chamber when said gel is liquid.

6. The device of Claim 5 which comprises (f) reagents to detect said analyte and (g) means for measuring a signal related to the amount of said analyte in said sample.

7. A method for amplifying and detecting a nucleic acid analyte using a sealable device, said method comprising the steps of:

(a) introducing a sample suspected of containing said nucleic acid analyte into a first chamber of said device preceding or following combining said sample with particles capable of binding said nucleic acid analyte,

(b) transporting said particles from said first chamber through a channel containing air or a liquefiable gel into a second chamber,

(c) sealing off said channel

(d) establishing fluid communication between said second chamber and a third chamber of said device,

(e) introducing into said third chamber a liquid medium for reagents for amplifying said nucleic acid analyte, when said liquid medium is not already present

(f) transporting said analyte into said third chamber,

(g) subjecting said medium to conditions for amplifying nucleic acids,

(h) examining said medium for the presence of amplified nucleic acid wherein the order of steps (e) and (f) may be reversed.

8. The method of Claim 7 wherein said particles are magnetic.

9. A kit for conducting an assay for a nucleic acid analyte, said kit comprising in packaged combination:

(a) the device of Claim 1

10. A kit for conducting an assay for a nucleic acid analyte, said kit comprising in packaged combination:

(a) the device of Claim 2 and

(b) particles capable of binding said nucleic acid analyte.

SUBSTITUTE SHEET (RULE 26)

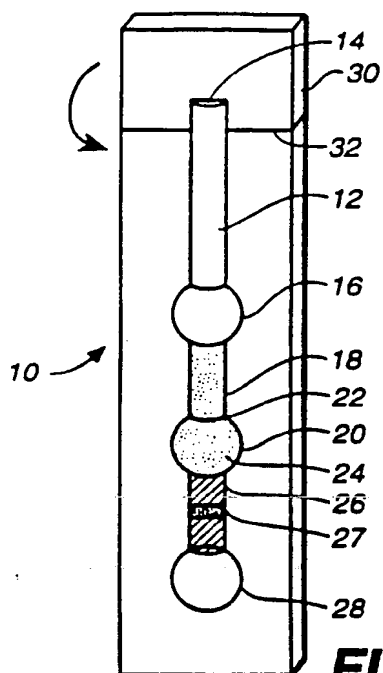


FIG._1

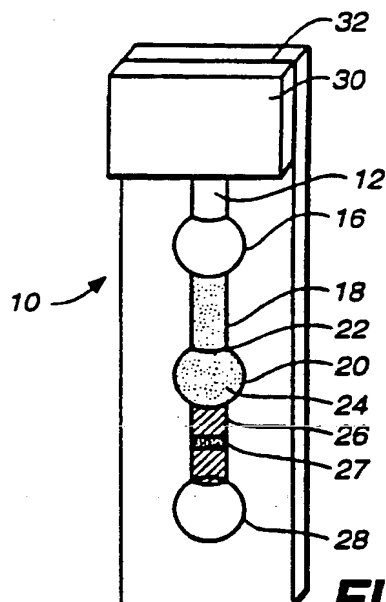


FIG._2

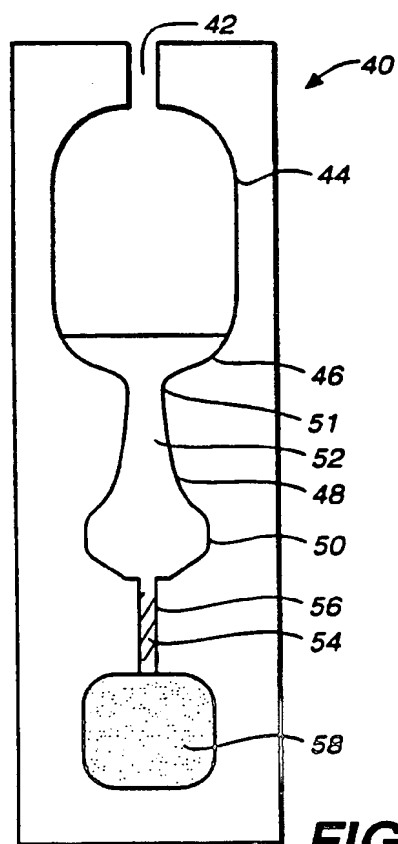


FIG._3

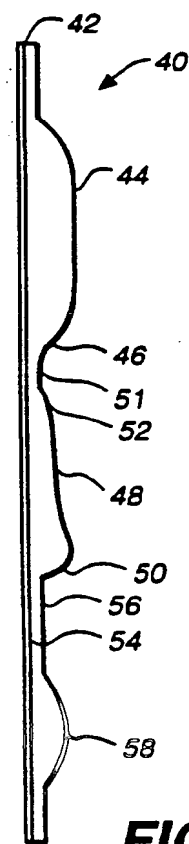
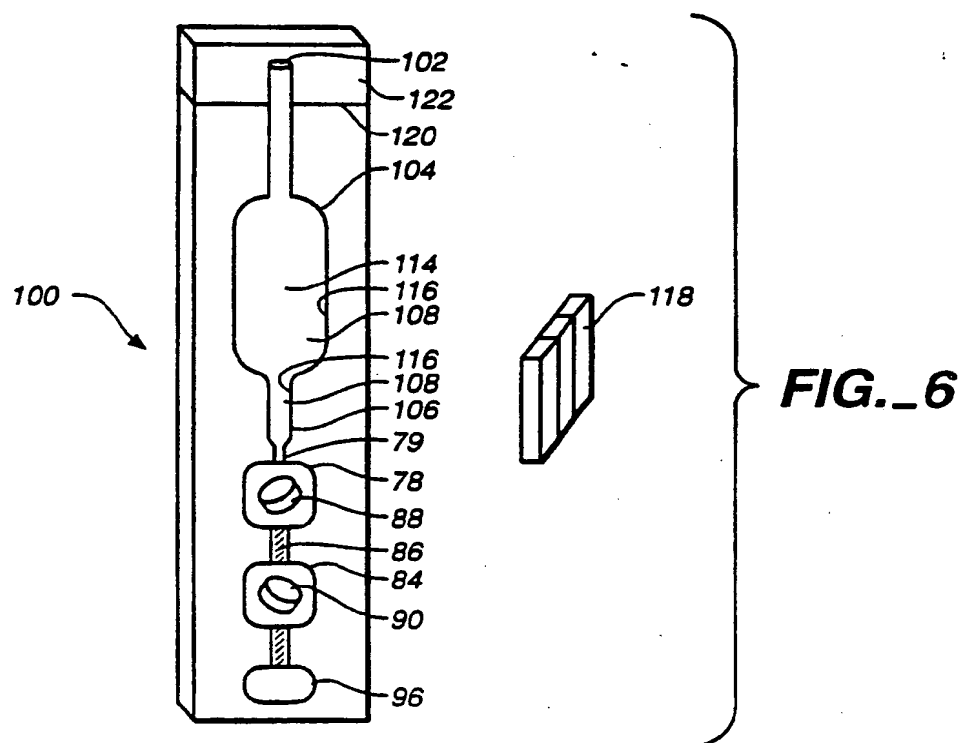
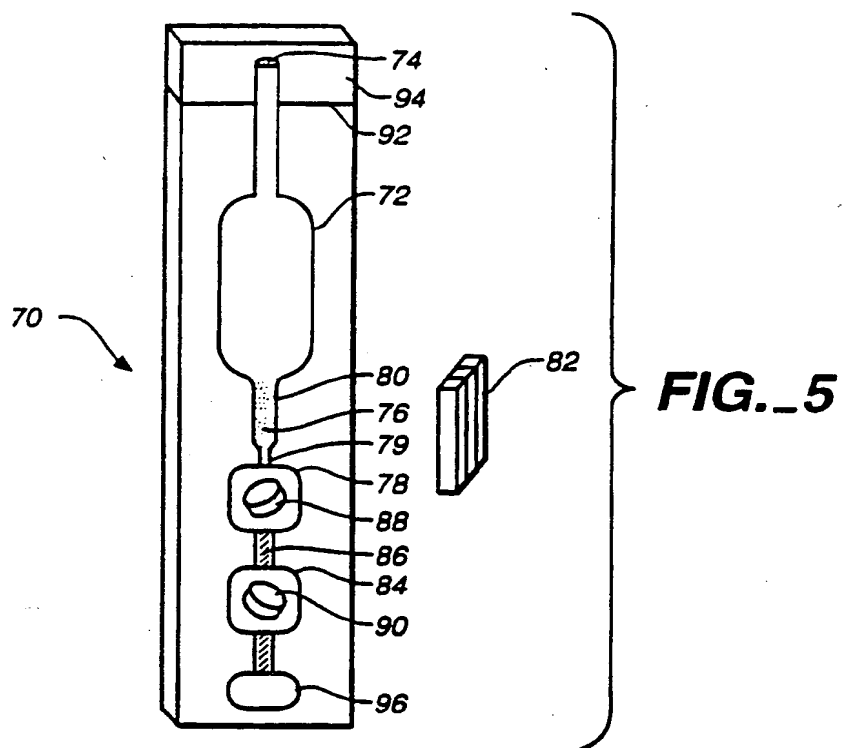


FIG._4

2 / 2



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 94/04849

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 B01L3/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC:

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No: |
|------------|---|-----------------------|
| Y | WO,A,89 05456 (BIOTOPE INC) 15 June 1989 see page 49, line 5 - page 52, line 15; figures 1,4 see page 24, line 8 - page 33, line 5 see page 41, line 10 - page 46, line 27 --- | 1-10 |
| Y | EP,A,0 381 501 (EASTMAN KODAK CO) 8 August 1990 cited in the application see column 12, line 2 - column 14, line 34; figures 1-5 --- | 1-10 |
| P,X | WO,A,93 22020 (AMOCO CORPORATION) 11 November 1993 see page 25, line 8 - page 23, line 22; figures 2-4 ----- | 1 2,4-10 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

12 September 1994

Date of mailing of the international search report

21.09.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Ex. 31 651 ext. nl.
Fax: (+31-70) 340-3016

Authorized officer

Bindon, C

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/04849

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO-A-8905456 | 15-06-89 | DE-A- 3882040 | 29-07-93 |
| | | DE-T- 3882040 | 30-09-93 |
| | | EP-A, B 0344276 | 06-12-89 |
| | | JP-T- 2502405 | 02-08-90 |
| EP-A-0381501 | 08-08-90 | DE-D- 69009510 | 14-07-94 |
| | | JP-A- 3007571 | 14-01-91 |
| | | US-A- 5229297 | 20-07-93 |
| WO-A-9322020 | 11-11-93 | NONE | |